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(71) Applicant *(for all designated States except US):* AMERSHAM INTERNATIONAL PLC [GB/GB]; Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* **:** FINLAN, Martin, Francis [GB/GB]; 7 Ballard Close, Aylesbury, Bucks HP21 9YU (GB). MALAN, Philip, Gordon [GB/GB]; Amersham International plc, Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA (GB).

(74) Agent: BOYDELL, John, Christopher; Stevens, Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London EC4Y 1LL (GB).

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(57) Abstract

There is described a surface plasmon resonance biosensor in which light is internally reflected at an internal surface of a transparent block (1). Against a surface (3) of the block is formed a layer (10) of silver overlayed by a layer (11) of sensitive material. The sensor monitors the change in refractive index of the layer (11) as the sensitive material reacts with a sample (not shown) in the area adjacent the sensitive material. This is achieved by creating the conditions necessary for surface plasmon resonance as the light is internally reflected. To do this, the angle of incidence at the internal surface must be correct and this is difficult to achieve without relatively sophisticated optics. In the invention the correct angle is achieved in a block having the form of a thin sheet by providing, on a surface (2) opposite surface(3) a diffraction grating (7) and passing the light from the light source (not shown) through this grating and thence taking just one of the thus diffracted beams entering the sheet (1) at a suitable angle. Other embodiments describe alternative ways of achieving the same object.

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"BIOLOGICAL SENSORS"

This invention relates to sensors for use in biological, biochemical and chemical testing and in particular to immunosensors used to monitor the interaction of antibodies with their corresponding antigens.

When antibodies are immobilised on a surface, the properties of the surface change when a solution containing a corresponding antigen is brought into contact with the surface to thus allow the antigen to bind with the antibody. In particular, the change in the optical properties of the surface can be monitored with suitable apparatus.

The phenomenon of surface plasmon resonance (SPR) can be used to detect minute changes in the refractive index of the surface as the reaction between the antigen and the antibody proceeds. Surface plasmon resonance is the oscillation of the plasma of free electrons which exists at a metal boundary. These oscillations are affected by the refractive index of the material adjacent the metal surface and it is this that forms the basis of the sensor mechanism. Surface plasmon resonance may be achieved by using the evanescent wave which is generated when a light beam is totally internally reflected at the boundary of a medium, e.g. glass, which has a high dielectric constant. The use of SPR in biological sensors has been known for some time, see for example our European patent application No. 0305109.

One problem with SPR-based sensors is the requirement for total internal reflection of a light beam travelling through a transparent medium such as glass at an internal surface of that medium. The physical requirements for such total internal

reflection dictate that the beam is incident at the surface at quite a shallow angle - typically less than 30^{o} with respect to the surface - and this can lead to problems in effectively arranging the optics. Various ways have been described: for example, in the aforementioned EP-A-0305109, a hemicylindrical lens is used to project light at the required shallow angle. Such an arrangement is effective, but in a practical device is undesirable because the lens is expensive, and can hardly be regarded as a throw-away item. To overcome this problem, the silver or other metallic layer necessary for SPR is applied not directly to the lens, but to a thin sheet of transparent material which latter is in turn optically coupled to the lens using a coupling fluid. Such a slab may take the form of a throw-away glass slide, such as a microscope slide or similar, or may take the form of a continuous or semi-continuous material which is moved to a fresh area in between each test.

Whichever of these is used, there is a need to index match, with a coupling fluid of appropriate refractive index, the transparent slab to the remaining non-replaceable optical components of the sensor. It is clearly better to have a system where the manipulation of index matching fluids is avoided.

In our EP-A-0343826, a continuous transparent membrane is used as the throw-away component mentioned above. Here, however, the membrane is supported not by a hemicylindrical lens as described above, but by a plane block of transparent material which, in the described embodiments, is rectangular in shape. In order to avoid the problem of refraction causing the point of total internal reflection to move about too much, thus reducing sensitivity, the incident light beam is taken via a reflecting surface whose shape is suitably shaped so that the effects of refraction are

eliminated, or at least reduced to an acceptable level.

Because of the continuous nature of the sensor described in EP-A-0343826, the problems of index matching the movable membrane with the fixed transparent block are even more acute, and a system which avoids the need for index matching altogether is clearly advantageous.

The present invention seeks to provide such a system. In accordance with the invention, there is provided a sensor for use in biological, biochemical or chemical testing, said sensor comprising a block of material transparent to electromagnetic radiation, a layer of metallic material applied to at least part of a first surface of said block, a layer of sensitive material applied to the metallic layer, means for introducing onto the sensitive layer so as to react therewith a sample to be analysed, a source of electromagnetic radiation, said radiation being incident on a second surface of said block, said second surface being so formed as to cause at least a portion of the incident ray to be transmitted into the block in a direction such as to result in total internal reflection, and hence surface plasmon resonance, at said first surface, and detector means positioned to receive the internally reflected beam, the arrangement being such that the characteristics of the surface plasmon resonance, as detected by the detector means, is dependent upon the reaction between the sample and the sensitive layer.

The invention is directed primarily towards those circumstances where the "block" of transparent material is relatively thin. Part of the object of the invention is to provide the apparatus with a non-expensive part which is thrown away or moved aside and replaced between each test. The other part of the

object of the invention is to achieve this without the need to use an optical coupling fluid (see above). The minimum requirements for a throw-away or replaceable part is to provide the metallic layer, and the sensitive layer. Since both of these layers are relatively thin, and are not readily self-supporting, clearly a third minimum requirement is a support layer. Physical requirements dictate that the support layer is positioned on the opposite side of the metallic layer from the sensitive layer and, since this is where the light is incident for SPR, the support layer must, in turn, be of transparent material. In previous apparatus, this transparent support layer has always been associated, from the optical point of view, with larger fixed optical components such as the aforementioned hemicylindrical lens and this immediately introduces the requirement of optical coupling fluids. What the present invention does, in essence, is to provide an apparatus in which the transparent support layer is used by itself to provide the internally reflected beam necessary for SPR.

Generally speaking, said first and second surfaces of the block will be generally parallel with one another and would then take the form of the major surfaces of a microscope slide or a film-like transparent membrane such as described in EP-A-0343826. However, the second surface has to be so formed as to cause the beam transmitted into the block to be in such a direction that surface plasmon resonance occurs at the apposite (first) surface. Various ways of achieving this are described herein. The methods may be broadly divided into three groups:-

1) The formation, on the second surface, of an optical diffraction grating onto which the incident beam from the radiation source is applied. The

zeroth order transmitted beam is substantially coaxial with the incident beam and is therefore of little value; however the higher order transmitted beams are directed into the block at ever larger angles with respect to the incident beam. Even the first order diffracted beam is likely to be deflected through a sufficient angle to cause total internal reflection at the opposite (first) surface and this will therefore be the one to use. If not sufficient, however, still higher order beams could be used. There is some loss of beam power in this method, but this can be compensated for either by using a suitably blazed grating, adapted specifically for the desired order, or by increasing the source power, or both.

2) The shaping of the second surface in such a way that that part of the second surface at which the incoming beam of radiation is incident is angled so as to transmit (rather than reflect) the incident beam, even though such incident beam is transmitted at an angle which will be internally reflected at the first surface. It will be appreciated that, where the first and second surfaces are generally parallel, there is a fundamental difficulty in applying to the block a beam of radiation which will, on the one hand, be at an angle sufficiently small with respect to the surfaces that total internal reflection will take place at the <u>first</u> surface (i.e. what is required) without causing, on the other hand, that same beam to suffer complete reflection at the second surface without ever entering the block. In addition to this it has to be remembered that the necessary angle for SPR is generally still smaller, thus making the problem more acute. In the present invention, the geometry of the second surface is such that, at the area of incidence, the angle of the first surface is such as to allow the incident wave to be transmitted.

At the same time, as will be explained in more detail below, the area of incidence can be shaped to provide a converging effect, so that a parallel incident beam may be converted into a converging "fan" beam, which has a focus on the first surface - for more detail of the fan beam concept, see our copending application EP-A-0305109.

3) The formation, on the second surface, of a roughened area onto which the incident beam is applied. The light transmitted into the block will be scattered over a range of angles with respect to the incident beam, and some of these angles will be sufficiently great as to result in total internal reflection on the opposite (first) surface. A true random roughness will cause this scattering to obey a cosine law of intensity with respect to the axis of the incident beam (Lambert´s Law) and will guarantee that some at least of the transmitted light will be bent through a sufficient angle to be totally internally reflected at the opposite surface.

Although the layer applied to the metal film is described herein as an antibody layer for use in immunoassays, it will be seen that any sensitive layer whose refractive index changes upon an event occurring can be used thus to provide a sensitive detector having a wide variety of applications in the fields of biology, biochemistry and chemistry. The material comprising the sensitive layer may be specific to a particular entity within the sample or may be non-specific (i.e. may interact with several species of entity within the sample). Examples of specific materials include recognition molecules such as the aforementioned antibodies which will specifically bind an analyte of interest within the sample, DNA/RNA probes which will bind with their complements in the sample liquid, or lectins, glycoproteins or enzyme

substrates, all of which are capable of recognising and binding with the other partner in a bimolecular recognition pair.

Examples of non-specific materials include hydrophobic materials, for example in the form of a monolayer of phospholipid-type molecules to capture amphipathic molecules, or hydrophilic materials which would capture polysaccharides. Indeed, it has been found that the surface of the metal layer itself can form an effective non-specific binding material. Silver or gold surfaces will bind proteins or polynucleotides such as DNA or RNA without the need for any further coating and, in this case, a separate sensitive layer is effectively dispensed with altogether, and the surface of the metal film used directly for the capture of entities within the sample to be tested.

The metal layer material is commonly silver or gold, usually applied by evaporation. The layer needs to be as uniform as possible in order to cater for minute movement in the point of incidence of the incoming beam. It is assumed that a structural metal film will give the best resonance and there are various ways in which the transparent block can be pretreated to improve the performance of the metal layer and in particular to control the natural tendency of such films to form discontinuous islands:-

1. Immersion in molten metal nitrates and other molten salts. This has the effect of introducing ions into the surface in a manner which can be structured and which can act as foci for island formation.

2. Ion bombardment to introduce nucleating sites. The removal of the more mobile ions has been demonstrated to reduce the thickness at which the evaporated film becomes continuous.

3. Electroless plating or electroplating over lightly evaporated films (0 to 100 angstroms thick). Electroless plated films survive to a greater thickness than evaporated films and could form more stable nuclei for subsequent coating.

4. Evaporating onto electroless plated films. The electroless plated films have a stronger tendency to an island structure and to bigger islands with greater spacing than evaporating films. This could be of advantage in tuning to light of a prescribed wavelength.

Coating performance can also be improved by:-

1. Controlling the surface temperature during coating. Using a higher temperature substrate to increase the island's size and the spacing between them and conversely.

2. Evaporating in the presence of a magnetic or electrostatic field or electron emission device to control the ion content of the vapour stream. The state of charge of the substrate is known to affect the island structure.

3. Controlling the angle of incidence of the evaporated vapour stream relative to the film surface. The mobility of the evaporated atoms and hence their ability to form bigger islands is greater when the momentum of the atoms relative to the film surface is increased.

In order that the invention may be better understood, several embodiments thereof will now be described by way of example only and with reference to the accompanying drawings in which:-

Figure 1 is a diagrammatic view of an SPR biosensor in which the incident light is deflected by means of a diffraction grating;

Figure 2 is a ray diagram showing the principle of operation of the embodiment of Figure 1;

Figure 3 is a view similar to that of Figure 1, showing an alternative embodiment;

Figure 4 is a version of the Figure 3 embodiment utilising multiple test sites;

Figure 5 is a perspective view of the Figure 3 embodiment, showing multiple test sites;

Figure 6 is a view similar to that of Figure 2, showing an embodiment utilising a random roughened surface;

Figure 7 is a view similar to Figure 1, showing an alternative embodiment;

Figure 8 is an enlarged view of part of Figure 7, illustrating the manner of constructing the shape of the input surface of the transparent block; and

Figure 9 is a diagrammatic side view of a "membrane" biosensor of the type suitable for use with the present invention.

Referring firstly to Figure 9, there will be described an example of the type of SPR biosensor with which the present invention may be used. It will be understood, however, that the invention is not limited to this particular biosensor.

The apparatus comprises a housing 34 having a hollow interior 35 in which is positioned a printed circuit board 37 on which is mounted the electronic circuitry associated with the apparatus. An aperture is formed in the top part of the housing, which aperture is covered by a support plate 31 of transparent material.

A radiation source 32 produces a collimated input beam 33 of electromagnetic radiation. The frequency of the radiation must be such as to result in the generation of surface plasmon waves and in practice will be within or near the visible region. Suitable sources include a helium neon laser or an infra red diode laser, but an ordinary light source,

with suitable filters and collimators, could be used.

The light beam 33 is applied to a mirror 36 which in turn directs the light onto a concave reflecting surface 38 and thence to the transparent support plate 31. The mirror 36 is driven by motor means (not shown), to rotate in an oscillatory manner between the limit positions shown by the solid and dotted lines. The result of this is that the light beam applied to the reflecting surface 38 scans backwards and forwards between the positions represented by the beams 22 (solid line) and 23 (dotted line).

Positioned in the top surface of the support plate 31 is a membrane in the form of a continuous film 24 which is movable from left to right in Figure 9 from a supply reel 25 to a take-up reel 26. In its simplest form, the membrane takes the form of a layer of flexible transparent material to which is applied a metal film layer for example of silver and a final layer of sensitive material, such as an antibody layer. The arrangement is such that the layers are in the order - transparent support plate 31 - flexible transparent layer - metal film layer - sensitive layer. Thus the sensitive layer is on the top when seen in Figure 9.

It will be noted that the flexible transparent layer lies directly against the transparent plate, possibly with an optical coupling fluid in between. Preferably the refractive index of the plate 31 is the same as that of the flexible transparent layer so that the two effectively act as a single transparent block, as far as light is concerned. Light incident from reflecting surface 38 enters the block and is incident on the metal film layer. The metal film layer causes the light to be internally reflected at a point 27 lying on the interface between the flexible

transparent layer and the metal film layer of the film 24. The internally reflected light passes out of the block, and is reflected off a further concave reflecting surface 28 to be incident on the sensitive surface of a light detector 29.

The reflective surface 38 has a shape which is such as to bring light incident thereon from a range of angles to a single point 27, despite the refraction which inevitably occurs when the light enters the transparent plate 31. Likewise, reflective surface 28 has a shape which is such as to bring light incident thereon from a range of angles to a single point at the sensitive area of detector 29.

Provided that the conditions are correct and, in particular, that the angle of incidence of the incoming beam at the interface between the flexible transparent layer and the metal film layer is correct, then surface plasmon resonance will result, causing a dip in the intensity of the internally reflected light as the angle of incidence of the incoming wave is scanned by the mirror 36. A picture of the whole dip can thus be built up by the detector 29 by relating the detector output, on a time basis, with the scanning movement of the mirror 36. This is carried out in the associated electronic circuitry.

The sensitive layer is one whose refractive index changes as it reacts with a sample, in the manner described above. This changes the angle of incidence at which surface plasmon resonance occurs, and thus the reaction of a sample with the sensitive layer can be monitored by observing the dip as the test proceeds. In order to carry out a test, it is simply necessary to place a sample to be tested on top of the sensitive layer in the area of the point 27 at which the light is incident, and observe the changes in the dip characteristics.

In the apparatus of the present invention, the plate 31 is dispensed with, and that surface of the film 24 onto which the light from source 32 is incident is treated in such a way as to cause the incident beam at point 27 to be internally reflected to cause the necessary surface plasmon resonance. This is achieved by impressing a pattern onto the surface. There are various possibilities, as will now be described.

Referring to Figure 1, there is shown a block 1 of material transparent to the radiation. The block 1 takes the form of a thin block of glass having parallel major surfaces 2, 3. An example would be a microscope slide. An incoming parallel beam 4 from a radiation source, such as source 32, is passed through a convex lens 5 which causes the beam to converge to a focus 6 at the upper, input, surface 2 of the block 1. The beam 4 is illustrated for clarity as just two ray lines representing the outer limits of the beam. It will be appreciated that the beam 4 is in fact a "solid" beam between the drawn lines.

That area of the surface 2 surrounding the focus point 6 is formed with an optical diffraction grating 7. In the simplest case shown, the grating is simply a series of parallel triangular section ridges extending across the surface 2 in a direction orthogonal to the plane of Figure 1. Such a grating will provide 4 series of diffracted waves: two reflected, and two transmitted into the block 1. Only one of the transmitted beams, reference 8, is shown, for clarity. This beam represents the first order diffracted beam. A similar first-order beam (not shown) is diffracted in the other direction. The beam 8 passes through the block 1, diverging as it does so, until it reaches the internal surface 3. At this surface, total internal reflection takes place,

and the reflected beam 12 emerges from the block at the edge 9 for detection in a photo sensitive or similar detector (not shown) such as detector 29.

Formed on the lower surface 3 of the block is a first layer 10 of silver overlayed by a second layer 11 of sensitive material. Provided the angle of incidence at surface 3 is correct, the arrangement shown will result in the generation of surface plasmons at the interface between the silver and the glass, in the manner described above. A sample (not shown) which is introduced into the area adjacent the sensitive layer 11 may or may not react with the sensitive layer, and any reaction which occurs between the two can be monitored by the surface plasmon resonance, using the information in the form of the internally reflected beam 12, as seen by the detector. The spread of angles in beam 8 incident on surface 3 ensures that the whole SPR dip can be monitored - for more detail, see EP-A-0305109.

It will be noted that, by the time the diffracted beam 8 has reached surface 3, it has spread somewhat and forms a solid, diverging, beam. This is a disadvantage in a biosensor which, for maximum sensitivity, requires that the area of interrogation is as small as possible. However, provided that the block 1 is relatively thin - typically 1 mm or less - then this should not be too much of a problem. For example, assuming a beam spread of 3° and a 1 mm thick block 1, the diameter of the area of interrogation will be 0.25 mm, which should give adequate sensitivity.

The shape of the ridges of the diffraction grating can be altered to tailor the grating output as required. In the present case the shape may be such as to give maximum output in the first order beams. Also, a crossed or "eggbox" grating may alternatively

be used, this giving a greater number of sets of beams emerging from the point 6.

Referring now to Figure 2, there is shown a computer-generated ray diagram which illustrates the principle of operation of the device of Figure 1. Where appropriate, the same reference numerals have been used and, as before, only a single one (reference 8) of the transmitted beams are shown. Although the beam is represented as a series of lines, it will be appreciated that it is, in fact, a solid beam of light, covering a spread of angles. Although not shown, it is assumed that, as in Figure 1, a diffraction grating is formed about the point 6.

For illustration the spread of the input beam 4 after being converged by lens 5 is shown as being great enough to provide a sufficient spread of the first-order diffracted beam 8 to give a situation in which, at surface 3, part 12 of the beam is totally internally reflected, as shown in Figure 1, and part 13 is transmitted into the medium below block 1, after undergoing refraction, as illustrated.

Reference is now made to Figure 3 which illustrates an embodiment similar to that of Figure 1. This embodiment is intended for use with a block 1 which takes the form of a continuous strip of transparent material, for example polymer, such as described in our copending application EP-A-0343826. To this end, the formations illustrated in Figure 3 will be repeated along the strip to enable either simultaneous multiple testing (see below), or movement of the strip from one test station to the next, or both.

At each testing station, the formations comprise firstly a convex protrusion 14 on surface 2, which protrusion carries the grating 7, and secondly a sawtooth-section groove 15 which defines a sloping

surface 16 through which the internally reflected beam 12 may emerge. The lens 5 is omitted.

The parallel incident beam 4 is applied directly to surface 2 in the area of the convex projection 14. The combined effect of the convex projection, and the diffraction grating 7 is to provide a first-order diffracted beam 8 which is converging. This beam is arranged to come to a focus 17 at the surface 3, and thus provides a fan beam, as referred to above, having a spread of angles to cover those necessary to cover the surface plasmon resonance dip. This arrangement thus avoids the problem of the somewhat larger area of interrogation found in the device of Figure 1, and should therefore give improved sensitivity.

The internally reflected beam 12 is able to emerge through the top surface 2 by virtue of the groove 15. Without this groove, the beam 12 would simply be internally reflected at the top surface 2 and would not therefore emerge from block 1. The surface 16 may be shaped to refract the beam in the manner required - perhaps to give a greater beam spread (which will give enhanced resolution at the detector) or to give no refraction at all.

Referring now to Figure 4, there is shown a block 1 in the form of a continuous strip illustrating the formation of a plurality of individual testing sites, of the type shown in Figures 1 and 3, along its length.

As shown in the enlarged inset, each testing site comprises features from both Figures 1 and 3: an input lens 5, in association with a planar grating 7 gives a diverging incident beam 8 to the interrogation area on surface 3, while a sawtooth groove 15 is used to allow the internally reflected beam to emerge from the upper surface 2 to be incident on a suitable

detector.

As illustrated, the device is set up for multiple testing of 4 separate samples (not shown), each brought into contact with a respective sensitive layer 11 associated with one of the testing sites. Separate photo sensitive, or similar, detectors can be used to receive the internally reflected light from each site, or a single large photo-sensitive array detector 18 can be used, as shown. If using a single detector 18, care should be taken that adjacent beams 12 do not overlap at the sensitive surface of the detector since this will lead to ambiguity.

It will be clear that the same continuous strip could be used for individual testing by indexing the strip along by one site between each test.

Up to now, it has been assumed that just a single testing site is present at each longitudinal position of the transparent continuous strip forming the block 1. In practice, the width of the strip is likely to be sufficient to enable multiple testing sites to exist across the width. By way of example, a version with four testing sites across the width is illustrated in Figure 5. The construction will be evident without detailed explanation - the same reference numerals are used where appropriate. It will also be apparent that the arrangement illustrated in Figure 5 could be used in association with that of Figure 4 to give an X-Y array of testing sites, the results from which could be detected by a single detector and analysed by an associated computer.

In Figure 5, the groove 15 is cut right across the width of the strip and so is used by all four testing sites across the width. The diffraction grating 7 may likewise extend right across the width, as a single grating, or may be formed as individual gratings, as shown. Separate light sources may be

used, or a single light source whose output is split to provide the individual separate input beams 4. In a still further alternative (not shown) the input beam 4 may be in the form of a planar sheet extending widthwise of the strip, and lens 5 a single cylindrical lens extending right across the width of the strip. Thus in this case, Figures 1 and 3 would show sections through the various parts, which extend above and below the plane of the drawing. If a Figure 3 arrangement is used, for example, the incoming fan beam 8 would in fact have a wedge shape in three dimensions, and the point of incidence on the surface 3 would be a line of incidence extending above and below the plane of the drawing. The metal layer 10 may be provided as a continuous strip across the width with individual "patches" of sensitive material applied for each of the four sites; alternatively, individual patches of both metal and sensitive material may be provided for each site.

A formation such as shown in Figure 5 may be repeated at equal intervals along the length of the continuous strip to enable a single such strip to be used for a plurality of tests by indexing between each test, or for multiple tests of an X-Y array, as mentioned above, or both.

It will be appreciated that, in the multiple testing versions illustrated in Figures 4 and 5, that other features taken from either of Figures 1 or 3 could be used in place of the features shown. In particular, the lens 5 in the versions of Figures 4 and 5 could be dispensed with, and the diffraction grating 7 instead formed on a convex protrusion, as illustrated in Figure 3.

Referring now to Figure 6 there is shown a computer-generated ray diagram similar to Figure 2 which is intended to illustrate the "scattering"

embodiment of the invention, referred to above.

In this embodiment, the input surface 2 of the block 1 is roughened, at least around the area where the input beam 4 impinges on the block. Although shown as a single line, the beam 4 would in practice be a solid beam similar to that described above. The beam 4 is shown impinging on the block at 90°, but this is not essential.

The effect of the roughened surface 2 is to cause the light to be scattered from the point 19 of impingement. Some light will be reflected, but this is not shown; the remainder is transmitted into the block and is scattered over a range of angles, as illustrated. Light will also be scattered to the left of the axis of incidence, but this is also not shown. At least some of the transmitted light will be bent through a sufficient angle at point 19 to be internally reflected when it reaches the surface 3; the remainder leaves the block 1 at the surface 3, as illustrated. The internally reflected light can be used in the manner described in more detail in Figure 1 to form the basis of an SPR sensor. It will further be appreciated that the teaching of Figure 6 could be applied to the embodiment of Figures 3, 4 or 5.

If the roughening on the surface 2 is random on a sub-wavelength scale then, by Lambert's Law, the transmitted light is scattered to have a cosine distribution of intensity in relation to the incident beam. Thus the intensity of the beam is highest along the axis of incidence (i.e. straight down in Figure 6), and reduces according to a cosine relationship as the viewer moves angularly away from the axis. That part of the beam which is incident at surface 3 at a suitable range of angles can be used to generate the surface plasmon which is used to monitor

the progress of the reaction (if any) between a sample and an adjacent sensitive layer.

Referring now to Figures 7 and 8, there is shown a further embodiment, comparable to Figure 4, showing multiple testing sites along the length of a block 1 in the form of a continuous strip of transparent material.

The top surface 2 of block 1 is shaped to allow the passage of both incoming and outgoing beams for each test site. This is achieved by forming, across the width of the strip, a series of parallel spaced grooves 20 which allow, in a comparable manner to the groove 15 in Figure 3, the internally reflected beam 12 to leave the upper surface 2. At the same time, each groove also allows the incoming beam 4 for the next adjacent test site to enter the block 1 through the top surface 2 without being substantially deflected.

The grooves 20 are defined by forming, on the surface 2, a series of parallel widthwise-extending spaced ridges 21. These ridges are convex in shape and, in the embodiment illustrated are in fact part-cylindrical. Thus the cross-sectional shape of each ridge 21, as seen in Figures 7 and 8, is an arc of a circle. Provided that the geometry is correct, this arrangement will bring to a focal point on lower surface 3 a parallel incident beam 4. By the same token, the internally reflected beam, diverging from the focal point on surface 3, will be converted into a parallel beam as it emerges from the upper surface of block 1, and continues as a parallel beam to the common detector 18. Thus lens 5 is not needed, and the incident beam is brought to a point or narrow line at surface 3, thus providing maximum sensitivity.

The calculation of the profile for the surface 2 is simply that of working out the shape which

focuses the incoming parallel beam 4 onto the internal surface 3. Figure 8 illustrates this for a continuous strip in the form of a polyester film of refractive index 1.65 where the angle of spread α of the incoming fan beam is chosen to be about 6° to span the surface plasmon resonance modes for typical biosensor environments at a silver-coated surface 3. Other surface structures which focus in this way are possible, with the near-cylindrical or spherical surfaces displaced by greater or lesser amounts, and with the surfaces through which the internally reflected beams exit made with other profiles. These would be matched to the requirements of the detector array 18.

It will be understood that, although shown for a multi-site device, the continuous film shown in Figure 7 could also be used for testing at a single station at a time, with indexing of the strip between tests. The strip could also be fabricated with a plurality of sites across the width, such as is illustrated in Figure 5.

The various shapes and treatments of the surface 2 of the block 1 in the above-described embodiments can be formed in various ways. It is now possible, on a mass production basis, to impress patterns onto transparent surfaces to the standards necessary to meet optical requirements. Conventional moulding or hot forming techniques may be used, assisted or replaced by, for example, chemically assisted excimer laser ablation of the surface.

CLAIMS

1. A sensor for use in biological, biochemical or chemical testing, said sensor comprising a block of material transparent to electromagnetic radiation, a layer of metallic material applied to at least part of a first surface of said block, a layer of sensitive material applied to the metallic layer, means for introducing onto the sensitive layer so as to react therewith a sample to be analysed, a source of electromagnetic radiation, said radiation being incident on a second surface of said block, said second surface being so formed as to cause at least a portion of the incident ray to be transmitted into the block in a direction such as to result in total internal reflection, and hence surface plasmon resonance, at said first surface, and detector means positioned to receive the internally reflected beam, the arrangement being such that the characteristics of the surface plasmon resonance, as detected by the detector means, is dependent upon the reaction between the sample and the sensitive layer.

2. A sensor as claimed in claim 1 wherein said block of transparent material comprises a relatively thin layer of transparent material in which said second surface is generally substantially parallel to said first surface, and wherein, on said second surface, or at least over that part thereof at which said radiation is incident, means are provided for deflecting at least a portion of said incident radiation by an angle sufficient to result in said total internal reflection at said first surface.

3. A sensor as claimed in claim 2 wherein said layer forms part of a continuous tape of transparent material which is indexed between tests to bring a fresh section of the tape into use.

4. A sensor as claimed in either one of claims 2 or 3 wherein said deflecting means comprises a diffraction grating operable to transmit and deflect light into the transparent layer as discrete beams, each defracted through a different angle and wherein at least one of the transmitted beams is incident at said first surface at an angle suitable to produce the surface plasmon resonance condition thereat.

5. A sensor as claimed in claim 4 wherein said grating is a blazed grating adapted to enhance the transmission of a beam having a particular desired order suitable for producing the surface plasmon resonance condition at said first surface.

6. A sensor as claimed in either one of claims 2 or 3 wherein said deflecting means comprises a roughened area of said second surface which is operable to scatter the light transmitted into the transparent layer over a range of angles including those which, at the first surface, will be incident at an angle suitable to produce the surface plasmon resonance condition thereat.

7. A sensor as claimed in claim 6 wherein said roughened area is randomly roughened to give a cosine distribution of transmitted radiation within said transparent layer.

8. A sensor as claimed in either one of claims 2 or 3 wherein said refracting means comprises a formation on said second surface, said formation being such that that part of the second surface at which the incoming beam of radiation is incident is angled with respect to the beam so as to transmit the incident beam into the transparent layer at an angle which, at said first surface, is suitable to produce the surface plasmon resonance condition thereat.

9. A sensor as claimed in claim 8 wherein said

part of the second surface has a convex shape operable to cause convergence of an incident beam within the transparent layer, the arrangement being such that the beam comes to a focus at the plane of said first surface.

10. A sensor as claimed in claim 9 wherein said formation comprises a series of parallel semicylindrical ridges of such size as to enable an incident beam to be transmitted into the transparent layer at one side of one ridge and to exit from the transparent layer, after the aforsaid total internal reflection at said first surface, at the opposite side of the next adjacent ridge.

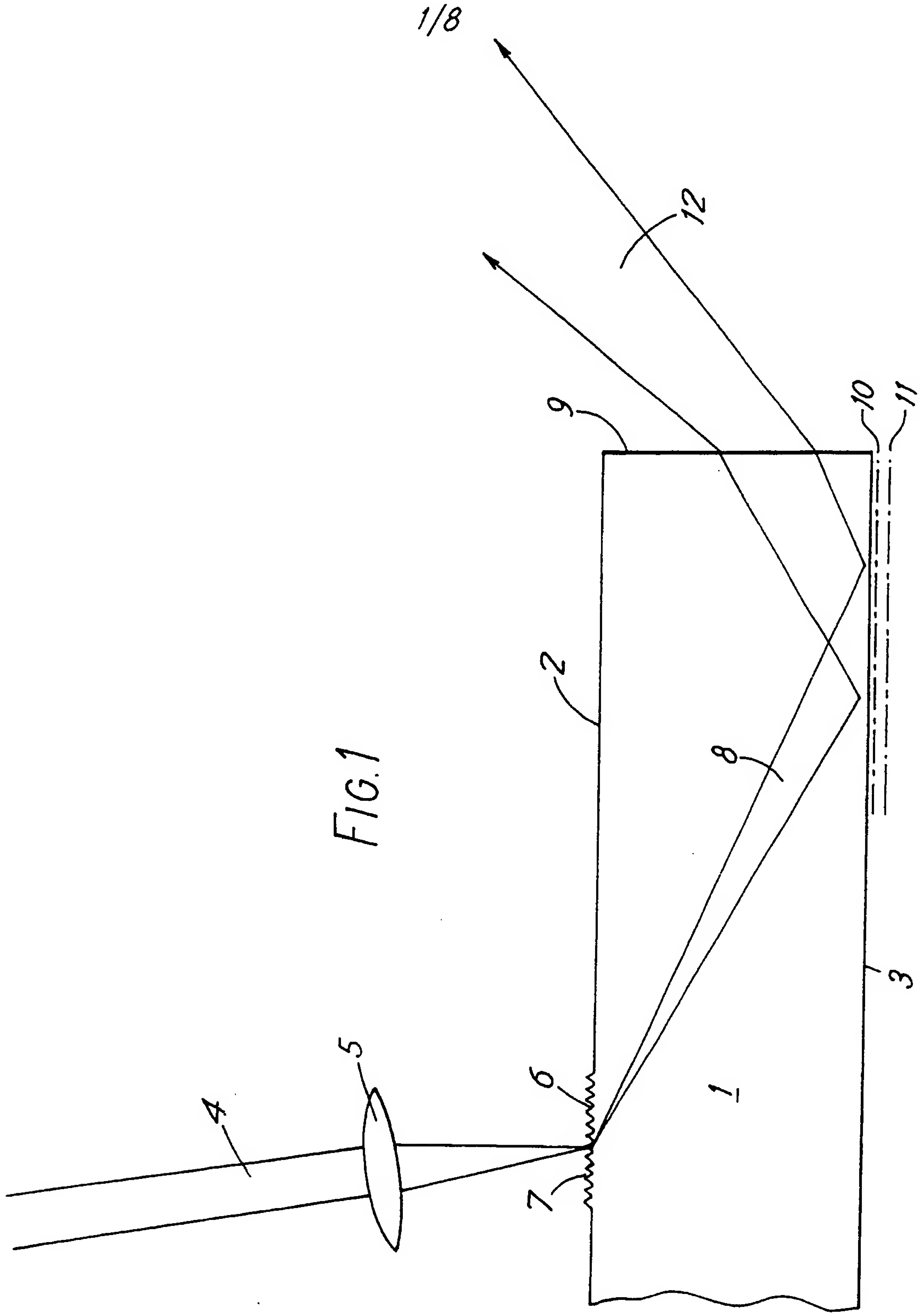

FIG.1

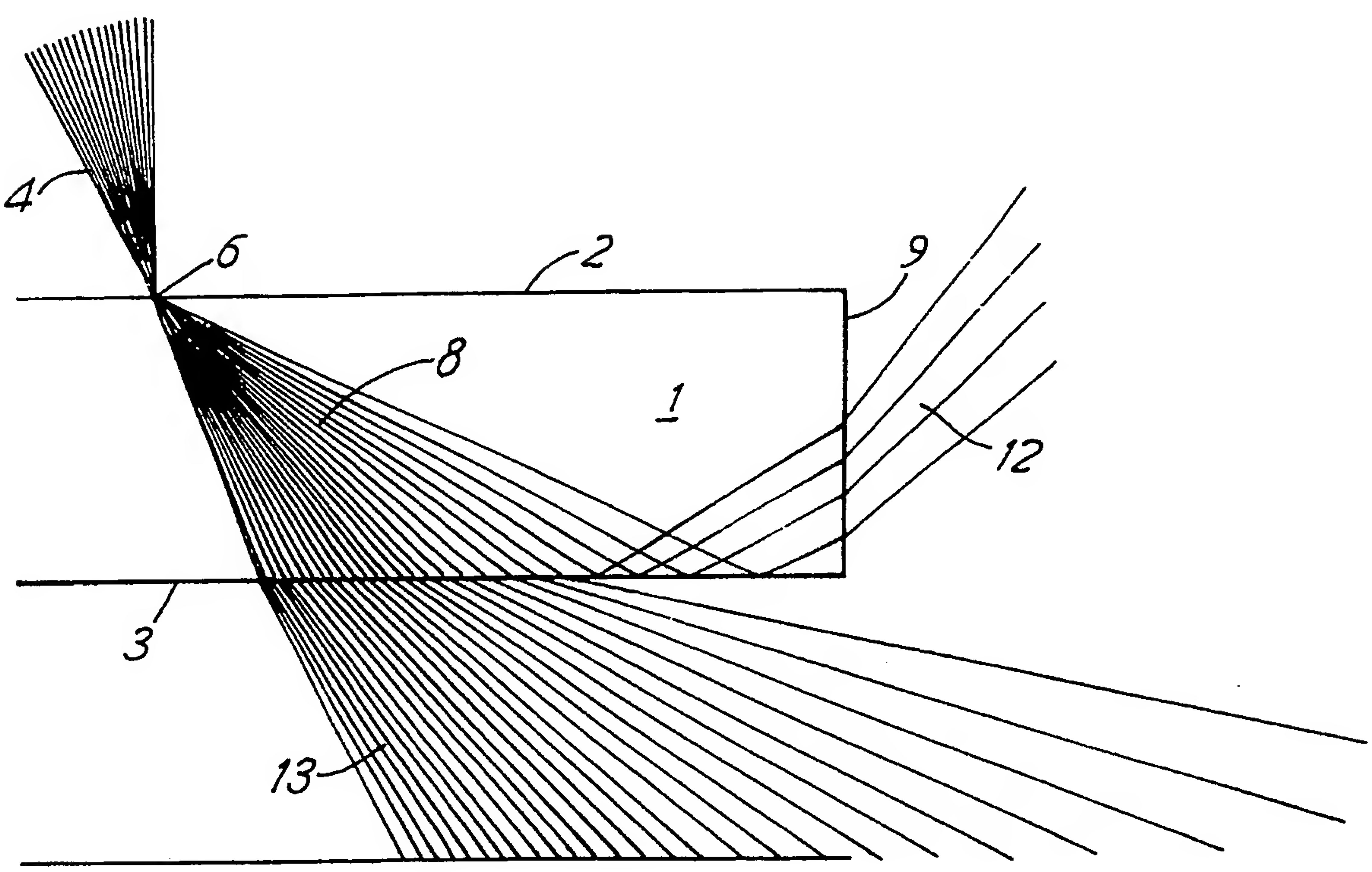

FIG. 2

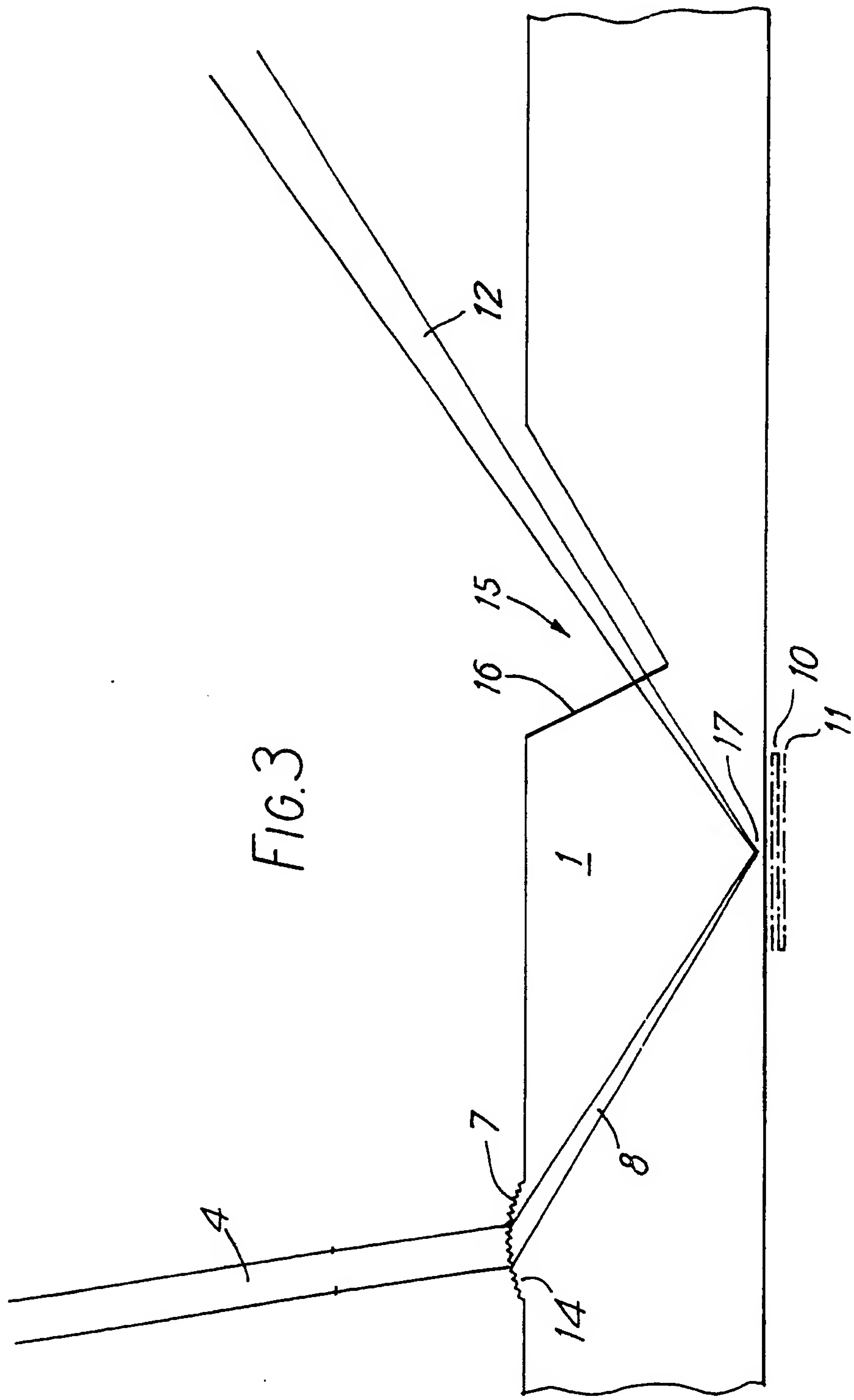

FIG. 3

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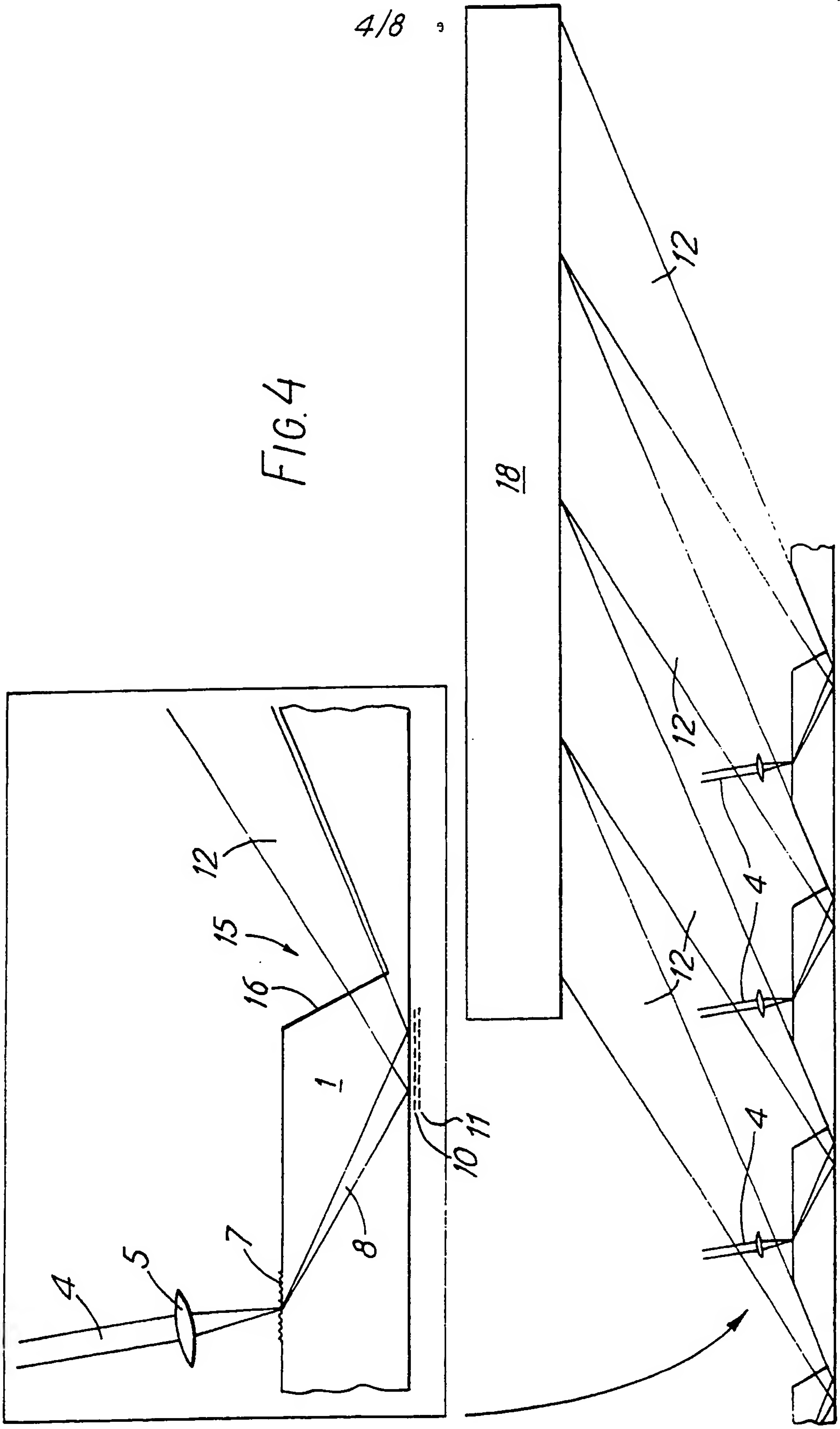

FIG. 4

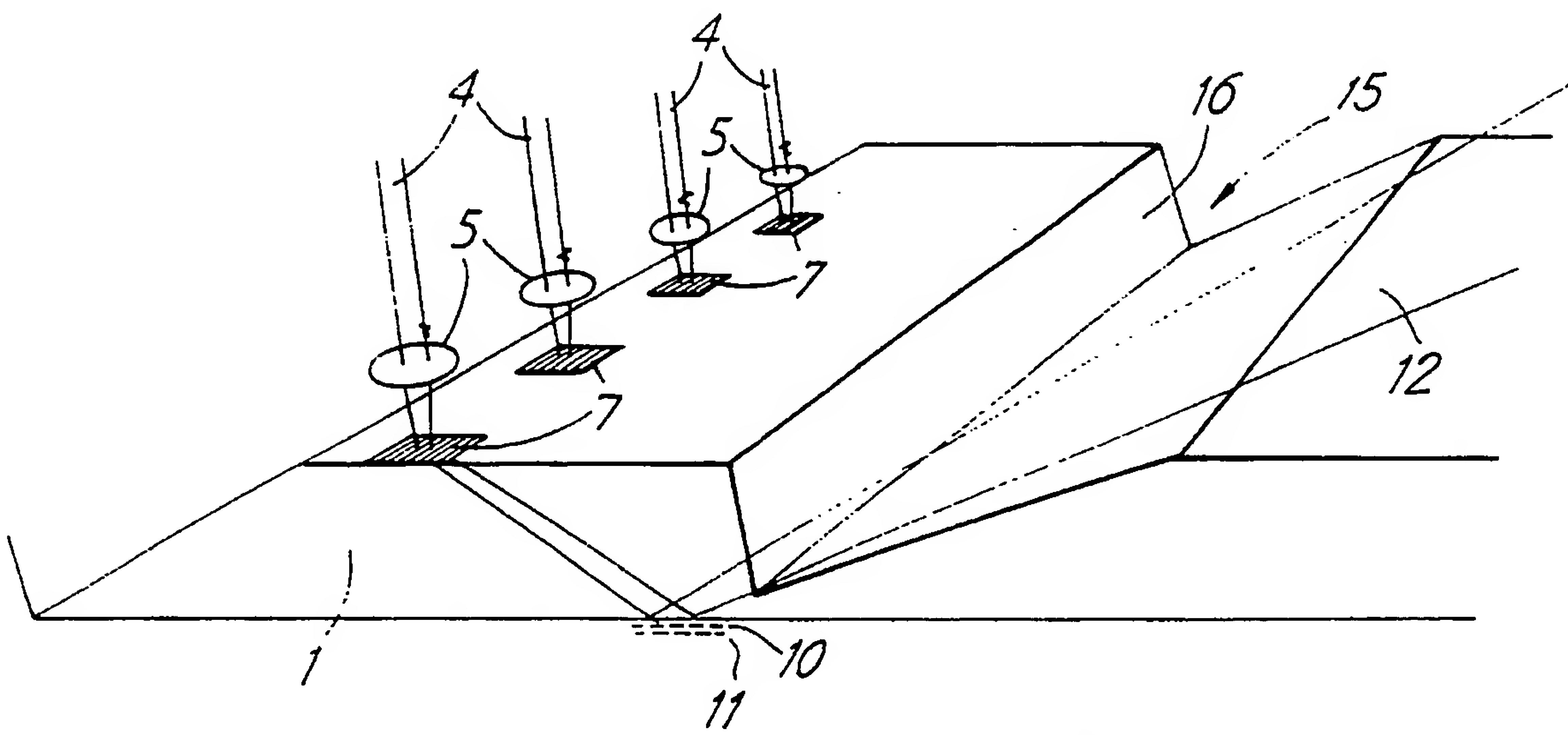

FIG.5

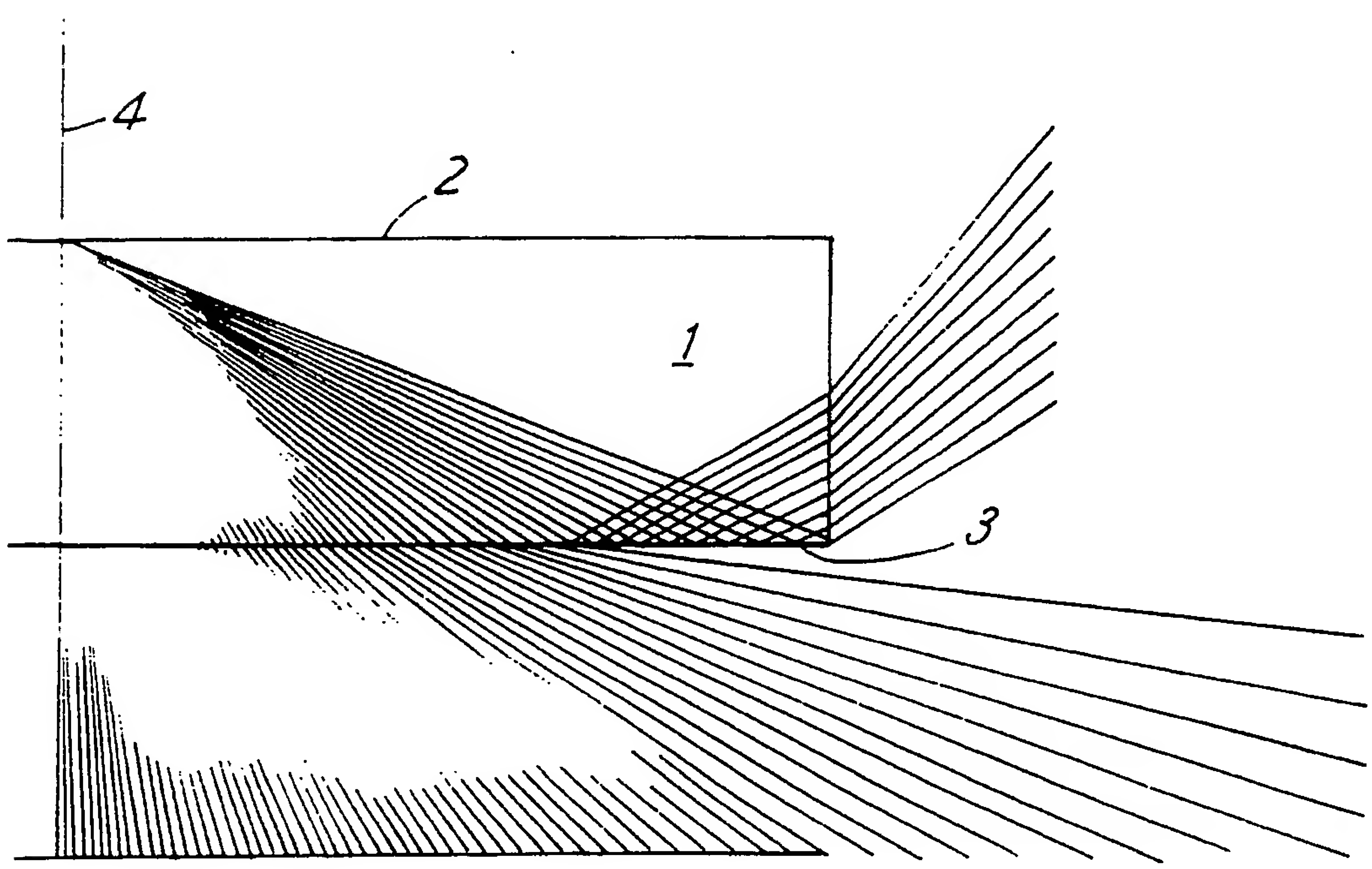

FIG.6

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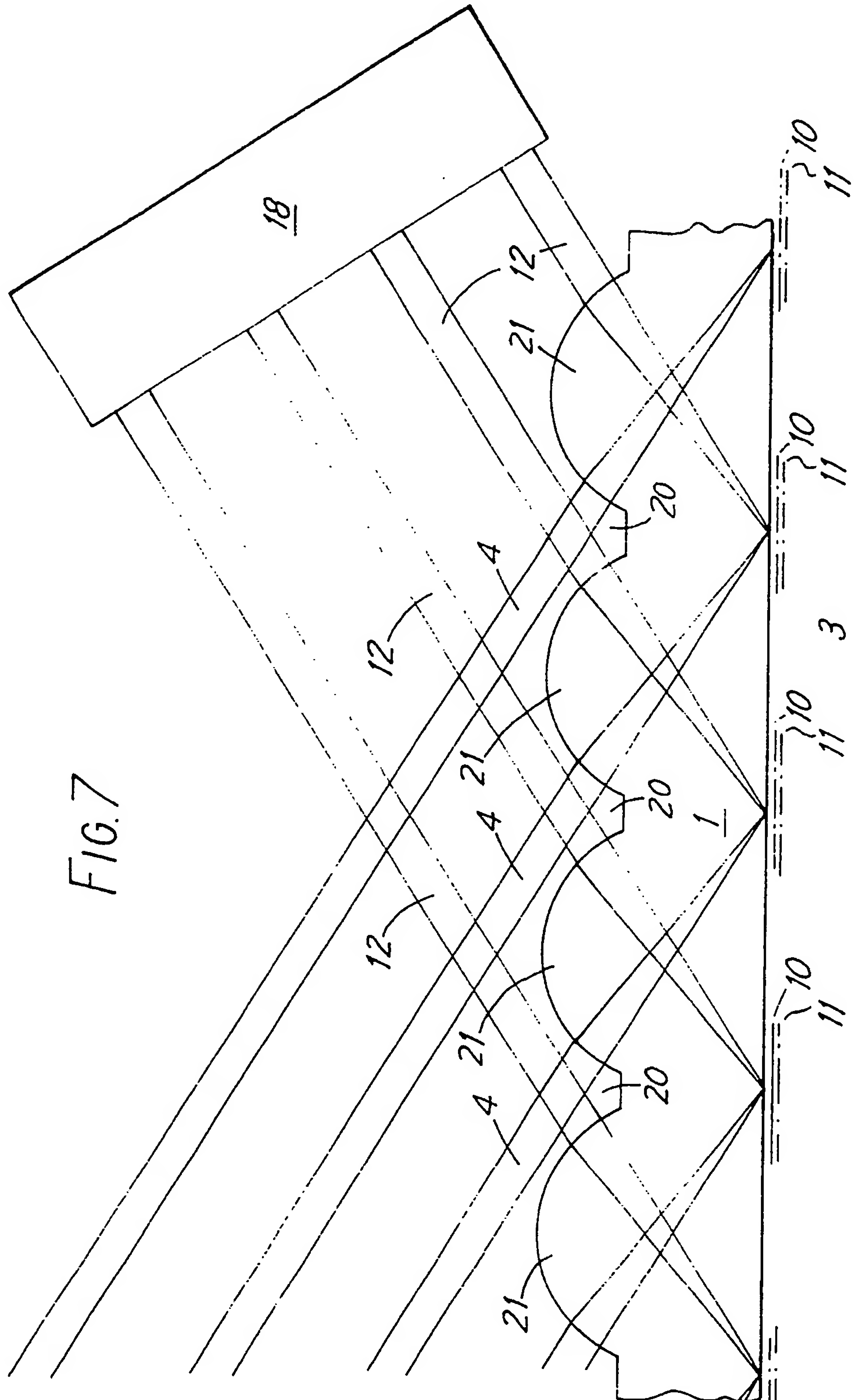

FIG.7

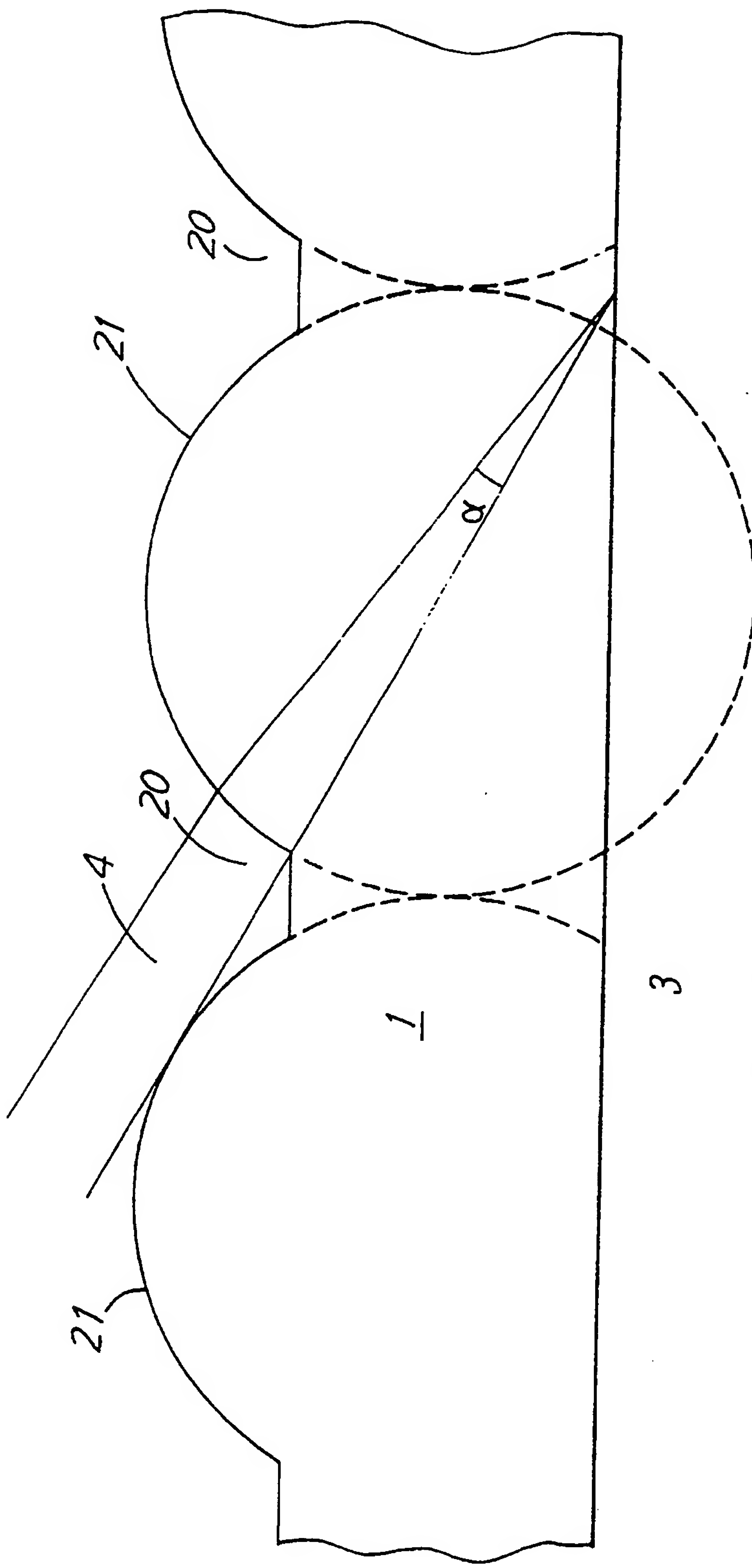

FIG. 8

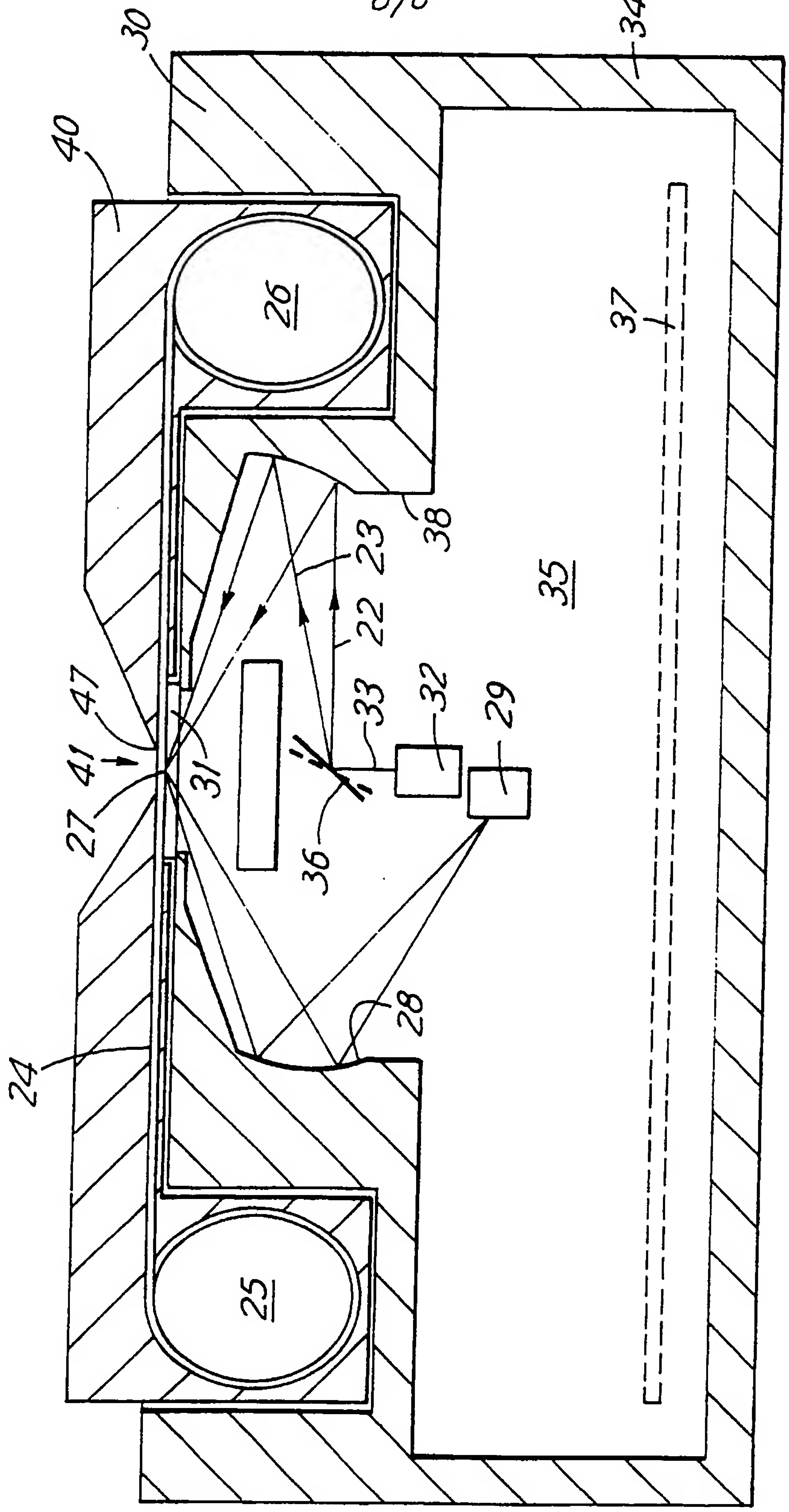

FIG.9

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01573

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N21/55

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0 343 826 (AMERSHAM INT.) 29 November 1989 see column 8 - column 13 ---	1
A	EP,A,0 305 109 (AMERSHAM INT.) 1 March 1989 see column 6 - column 8 ---	1
A	GB,A,2 185 308 (STC PLC) 15 July 1987 see page 1 - page 2 ---	1
A	EP,A,0 341 928 (AMERSHAM INT.) 15 November 1989 see column 11 - column 13 ---	1
A	WO,A,8 909 394 (ARES SERONO RESEARCH) 5 October 1989 see page 8 - page 10 ---	1

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 DECEMBER 1991	13 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	BOEHM C.E.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101573 SA 51326

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 11/02/92
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		US-A- 5023053	11-06-91
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		AU-A- 2110988	23-02-89
		JP-A- 1138443	31-05-89
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GB-A- 2185308	15-07-87	None	
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		AU-A- 3460989	16-11-89
		EP-A- 0341927	15-11-89
		JP-A- 2103469	16-04-90
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		AU-A- 3360389	16-10-89
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		JP-T- 2504313	06-12-90

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82